Genetic Structure of Marine Invertebrate Stocks in California State Marine Ecological Reserves

Ronald S. Burton

Scripps Institution of Oceanography, UCSD
9500 Gilman Drive
La Jolla, California 92037

Marine Ecological Reserves Research Program

Project Number 1-M-N
June 1, 1997 – August 31, 1999

Disclaimer

The mention of commercial products, their source or their use, in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the state of California or its agents.

Summary

A primary goal of the California Marine Ecological Reserves is to protect and enhance coastal marine resources. By protecting selected populations of marine organisms, the reserves will presumably benefit extended areas of coastline as the protected populations provide offspring that may disperse into neighboring habitat. The effectiveness of the reserves as a management tool, therefore, depends on the extent to which populations within the reserves are self-sustaining and/or capable of providing recruits to adjacent habitat patches. Although many marine organisms have impressive dispersal capacities (usually as planktonic larval stages), it is generally unknown if the dispersal of these organisms is sufficient to repopulate exploited, nonreserve coastline. This project employed genetic analyses to assess levels of population differentiation and dispersal abilities of three marine invertebrates that represent important ecological and commercial resources.

Target organisms included two species of abalone (the black, *Haliotis cracherodii*, and the red, *H. rufescens),* and one species of sea urchin (the purple, *Strongylocentrotus purpuratus*). The original plan to include a second urchin species (the red urchin, *S. franciscanus*) was abandoned because samples could not be obtained from the reserves. Although all these species rely on planktonic larvae as a means of dispersal, larval duration among abalone species is shorter than that observed in urchins (1–2 weeks as opposed to 4–8 weeks), suggesting more restricted dispersal and presumably reduced population intermixing in abalone. Abalone fisheries, once strong, have faced serious population declines in Central and Southern California. Population declines have been especially severe among black abalone in Southern California. Red abalone have already been the focus of reseeding efforts and it has been recently suggested that implanted hatchery animals have had a significant impact on population genetic structure (Gaffney et al. 1996). The research conducted under this award addressed the urgent need for baseline population genetic studies to understand dispersal among natural abalone and urchin populations. This information can be used to better assess the impact of the reserves, reseeding programs, and other management practices.

Two studies were completed under this award, and are now published (see Burton and Tegner 2000; Hamm and Burton 2000). A third study provides data concerning the urchin populations in the Vandenberg and Big Creek Ecological Reserves, and will be published along with supporting data from other sites at a future date. A description of the work and the results of the three studies will be summarized separately in the following parts.

Part One

Population Genetics of Red Abalone (*Haliotis rufescens*)

with reference to dispersal and the success of hatchery outplants (Burton and Tegner 2000)

Introduction

Although we were unable to obtain red abalone samples from the reserves themselves, populations included in the study neighbored the Vandenberg and Punta Gorda Ecological Reserves.

As marine fisheries are depleted, efforts to enhance natural stocks via outplants of hatchery-reared seed continue to grow (Munroe and Bell, 1997). Because of their high market value and depressed natural populations (Shepherd and Brown, 1993; Schiel, 1994), abalone outplanting has attracted considerable interest in various parts of the world. The California Department of Fish and Game and the California Sea Grant College Program have conducted a variety of seeding experiments with hatchery-reared juvenile abalone in California (reviewed by Tegner and Butler 1989; Tegner 2000). These experiments generally have not produced encouraging results. However, Gaffney et al. (1996) recently reported that the genetic composition of red abalone, *H. rufescens*, collected from San Miguel Island in 1992 strongly suggested that the animals were of hatchery origin. They concluded that the animals were derived from an outplant experiment conducted at Tyler Bight on San Miguel Island in 1979. A total of 42,431 red abalone seed ranging in size from 10 to 34 mm were released in August and October 1979. Unfortunately, most of the seed for this experiment came from a hatchery with recirculating seawater that caused severe shell erosion; many of the seed were fragile and shiny, making them susceptible to high predation pressure (Tegner and Butler 1989).

Despite early indications that the Tyler Bight outplant was unlikely to be successful, Gaffney et al. (1996) suggest that it had, in fact, supported a significant fishery at San Miguel in the 1980s. Their inference regarding the outplant success was based primarily on two observations: (1) allelic frequencies at two allozyme loci (of four surveyed) in the San Miguel sample were significantly different from those observed in natural populations sampled in both Northern and Southern California; and (2) all individuals sampled at San Miguel were heterozygous for the same two alleles at the GPI locus. This suggests they originated from a hatchery rather than from a natural population, which would likely show Hardy-Weinberg expected genotypic propor-

tions. These results and conclusions have important implications. First, the results suggest that a 1979 outplant had dramatically altered the genetic composition of a natural population sampled 13 years later. Second, the fact that all animals collected at San Miguel in 1992 were GPI heterozygotes suggests that this population had experienced no natural recruitment to the fishery between 1979 and 1992 (since at least half the natural recruits would be expected to be homozygotes at GPI). Finally, the report suggests that red abalone outplanting has been successful and might, therefore, merit considerable funding support.

Given the importance of correctly assessing outplanting success, we initiated follow-up investigations of populations of *H. rufescens* on San Miguel Island (SMI).

Materials and Methods

Sample collection: *H. rufescens* were sampled from Tyler Bight to Crook Point on the south side of SMI in March 1999. This is the same region sampled by Gaffney et al. (1996). All of the sampled animals were sport legal (178 mm) size or larger; some were commercial size (197 mm). Sampling was nondestructive; divers detached each abalone from the substrate, pinched off an epipodial tentacle with a locking forceps, and returned the animal to the rock substrate. After surfacing, divers removed the samples from the forceps, placed them in individual 1.5 ml microfuge tubes, and froze the tubes in liquid nitrogen for shipping. Once in the laboratory, samples were stored at –80° C. Samples of epipodial tissue from two Northern California populations, Horseshoe Cove (HSC, southern Sonoma County, at Bodega Bay) and Shelter Cove (SH, southern Humboldt County), were kindly provided by C. S. Friedman (Bodega Marine Lab[1]).

Protein electrophoresis: Electrophoretic analyses were conducted as described in Kordos and Burton (1993), using 6% polyacrylamide slab gels (0.75 mm thick) and enzyme staining by standard recipes (Harris and Hopkinson 1976). The enzymes examined were: aspartate aminotransferase (the faster migrating of two loci, AAT1, EC 2.6.1.1 = GOT), phosphoglucomutase (PGM, EC 5.4.2.2) and glucose-6-phosphate isomerase (GPI, EC 5.3.1.9). Allozyme data were analyzed using the software package POPGENE 1.21 (Yeh and Boyle 1997). Contingency tables (G-tests) were used to compare allelic counts for population differentiation and for tests of deviations from Hardy-Weinberg genotypic proportions. Levels of population subdivision were calculated using Wright's standardized variance in allelic frequencies (F_{ST}); significance of F_{ST} was determined using the methods described in Waples (1987).

Mitochondrial DNA analyses: DNA was extracted from approximately 6 mg of tissue by incubation in 200 µl of 10% chelating resin (Sigma) for

[1]Now at the University of Washington, Seattle.

8 min. at 100° C. A 580 bp fragment of the mitochondrial gene cytochrome oxidase subunit I (COI) was amplified using the polymerase chain reaction (PCR). Thermal cycle settings were: 3 min. initial denaturation at 94° C, 35 cycles of 95° C (1 min.) 50° C (1 min.) 72°C (1.5 min.), with a 7 min. final extension at 72° C. Abalone specific primers were designed by Metz et. al. (1998): forward: 5' TGATCCGGCTTAGTCGGACTGC 3', reverse: 5' GATGTCTTGAAATTACGGTCGGT 3'. PCR products were visualized on 2% agarose TAE (tris-acetate-EDTA) and prepared for sequencing using the QIAquick PCR Purification Kit (Qiagen, Inc., Chatsworth, CA) and sequenced on an Applied Biosystems 373 Autosequencer using the Big Dye Terminator RR chemistry (Applied Biosystems) following manufacturer's protocols. Sequences were aligned with Sequencher 3.1 (Genecodes Corp.) and analyzed using PAUP 4.0b2 (Swofford, 1999) and DnaSP Version 3.0 (Rozas and Rozas, 1999).

Results

Sample sizes and observed allozyme frequencies for the 1999 population samples are presented in Table 1; data from Gaffney et al. (1996) are included for comparison. First, considering only the 1999 samples, no significant deviations from Hardy-Weinberg expected genotypic proportions were observed at any of the three loci in any of the three sampled populations. Marginally significant genetic differentiation was apparent at the AAT1 locus (contingency table test of homogeneity of allelic counts, $X^2 = 12.51$, df = 6, p = 0.051); no significant population heterogeneity was observed at the GPI or PGM loci. Calculated F_{ST} values both at the individual loci (0.016, 0.006, and 0.013 for AAT1, GPI and PGM respectively) and averaged across loci (0.012) did not differ significantly from zero.

When the new SMI-99 data were compared to SMI-92 data, significant differentiation was observed at two of the three loci ($X^2 = 40.8$, $p < 0.001$ for GPI, $X^2 = 14.0$, $p < 0.001$ for AAT1). The AAT1 differentiation cannot be attributed to a hatchery source for SMI-92 animals because SMI-92 does not differ from the allelic frequencies observed in the SC-79 sample. In contrast, the SMI-92 GPI frequencies differ from those observed in all natural population samples. Again, the most striking observation regarding the SMI-92 GPI data concerns the total lack of homozygotes; all 1999 samples were dominated by homozygotes as expected from the observed allelic frequencies.

COI sequences (484 bp) were obtained from 20 animals each from SMI and SH, and 21 animals from HSC (Table 2). Fifteen nucleotide sites were variable among the 61 sequences; all substitutions were synonymous. Only seven nucleotide sites were informative; ignoring variation at uninformative sites (i.e., singletons), there are seven different composite haplotypes (Table 2). All haplotypes observed more than twice were present in all three populations. Haplotypic diversity (including singletons) in the total sample was high (0.83) and did not vary significantly across populations (extremes of 0.81 ±

0.07 at SMI to 0.87 ± 0.04 at HSC). Nucleotide diversity (π) was also similar across populations, only ranging from 0.0042 ± 0.0005 at SH to 0.0046 ± 0.0009 at SMI. No significant differences in haplotypic frequencies were observed among populations and none of the pairwise F_{ST} analogues calculated by the DnaSP program (including Nei 1982; Lynch and Crease 1990; Hudson et al. 1992) were significantly different from zero.

Discussion

An experimental abalone enhancement program was established in California after a precipitous decline in landings began in the late 1960s (Tegner 2000). Seeding experiments in California included three abalone species in a range of sizes, planting densities, and techniques. Most of the studies were evaluated by extensive field surveys (although the data from the Tyler Bight experiment on San Miguel Island have not been analyzed). In each case, the estimated one- or two-year survival rates based on the recovery of live seed was 2.8% or less.

Although there are good reasons not to use rare genotypes in stock enhancement programs, lack of genetic tagging makes long-term evaluation of such efforts difficult. To date, no California outplants have been deliberately genetically tagged. The genetic anomalies reported by Gaffney et al. (1996) for their SMI-92 sample, however, are certainly suggestive of hatchery origin: both unusual allelic frequencies and large deviations from Hardy-Weinberg genotypic proportions could be indicative of a cohort of animals derived from few female broodstock. However, in this particular case, the genetic signal was actually stronger than expected—it is difficult to construct the hatchery and natural population conditions that would produce a sample of 60 animals with 100% heterozygosity at GPI. This observation initiated our interest in resampling the SMI population. As detailed above, our SMI-99 sample shows no evidence of the genetic anomalies that led Gaffney et al. (1996) to conclude that their 1992 SMI animals were the result of a 1979 hatchery outplant. In fact, based on the scored marker loci, the SMI-99 sample closely resembles that reported for 1979 (pre-outplant) Southern California and 1992 Northern California red abalone.

There are at least two possible explanations for the differences in the two SMI data sets: (1) there was a dramatic change in the genetic composition of the San Miguel population in the seven years between sampling dates (1992–1999), or (2) GPI was mis-scored in Gaffney et al. (1996), perhaps due to sample degradation or laboratory artifact. Since we are unaware of frozen archived tissues from SMI (dating from 1979–1992) that could be used for allozyme analyses, we cannot directly test either hypothesis. We raise the latter possibility because the data, as the authors admit, are difficult to explain. The probability that a hatchery could produce a set of all heterozygous offspring at GPI is small. Assuming that the hatchery broodstock came from California, the probability of such a single mating of alternate homozygotes is less than

0.03 (Gaffney et al. 1996). Furthermore, as Gaffney et al. note, the other loci studied indicate that the SMI cohort was the result of multiple parental pairs; the probability that several males were one homozygous type and several females were the other is negligible—$(0.03)^2 < 0.001$ for even two such pairs. Furthermore, the Gaffney et al. results suggest that essentially all red abalone sampled at SMI in 1992 were outplants; i.e., there was no natural recruitment to the fishery in this population (since over 75% of animals in natural populations are GPI homozygotes). To account for the difference between the 1992 sample and our 1999 sample, the frequency of outplants at the site must have gone from near 100% to near zero in the seven-year period. Given that the outplants must have been extremely successful for 13 years in order to dominate the 1992 sample, this seems improbable.

An alternate explanation is that some laboratory artifact led to mis-scoring GPI in the SMI-92 sample. This possibility is raised by a similar situation in the literature. Cole and Morgan (1978) scored nearly all individuals of the blue crab, *Callinectes sapidus*, collected from two bays in Maryland, as GPI heterozygotes and reported frequencies of 0.45 and 0.55 for the two observed alleles. This level of GPI heterozygosity is inconsistent with other surveys of allozyme polymorphism of Atlantic coast *C. sapidus* (e.g., McMillen-Jackson et al. 1994), which have found that GPI is only weakly polymorphic over the entire Atlantic range of the species. Here again we cannot prove an error in scoring occurred, but the large deviations from Hardy-Weinberg genotypic proportions in the suspect samples warrant cautious interpretations. It seems possible that GPI allozymes are susceptible to producing anomalous banding patterns on gels, perhaps due to partial degradation during storage under suboptimal conditions.

Our mtDNA sequence data further support our conclusion that the SMI-99 sample lacks a genetic signature of outplant success. If red abalone at SMI derive from hatchery stocks, one might expect some reduction in mtDNA diversity if the number of broodstock were limited (as suggested from the GPI data). No evidence was found for such a reduction; mtDNA diversity at SMI is presently indistinguishable from that observed at HSC and SH, with two Northern California sites harboring robust natural populations of *H. rufescens*.

Irrespective of the explanation for the genetic anomalies observed in the SMI-92 sample, the data presented here indicate that no genetic signature of outplants exists in the more recent SMI-99 sample. This, of course, does not mean that the outplant operation was unsuccessful. Since the outplants were not deliberately marked, there is no *a priori* reason to expect a genetic signature. Our conclusions, then, are simple: (1) there is no genetic evidence, from our recent resampling of *H. rufescens* from the south side of SMI, that the 1979 outplant was successful; (2) previous genetic data reporting outplant success at this site is rather suspect; and (3) even if the outplant was successful up to the 1992 sampling, our data suggest that the success of the operation

was limited to the grow-out period of the original outplants. Had the outplants successfully reproduced and experienced some local recruitment, the frequency of the GPI^C allele should have shown some increase over time. Instead, the SMI-99 sample had the lowest frequency of this allele yet observed in a natural California population.

Finally, it should be noted that existing genetic data from the present study as well as that in Gaffney et al. (1996), Metz et. al. (1998), and Kirby et al. (1998) suggest that there is not strong population divergence in *H. rufescens* over its California range. The allozyme results presented here are consistent with previous allozyme studies in abalone that has revealed only low levels of intraspecific population divergence (see review by Withler 2000). However, other types of genetic markers have revealed considerable variation in population structure in some abalone species. For example, Jiang et al. (1995), found fixed differences in mitochondrial DNA haplotypes between neighboring populations (35 km apart) of Taiwanese abalone, *H. diversicolor*. The mtDNA data presented here for three *H. rufescens* populations spanning approximately 600 km of coastline suggests that this species does not exhibit such pronounced genetic differentiation. However, further studies examining additional populations and employing more genetic markers are needed to more thoroughly assess the genetic structure of this economically and ecologically important species.

Part Two

Population Genetics of Black Abalone (*Haliotis cracherodii*)

along the central California coast (Hamm and Burton 2000)

Introduction

Because knowledge of dispersal and population structure is central to the development of abalone conservation and management plans, population genetic studies of the black abalone, *H. cracherodii*, were initiated along its present range on the Central California mainland. Populations of the formerly abundant, *H. cracherodii* have all but disappeared in Southern California. In part, the population declines can be attributed to heavy fishing pressure (Morris et. al., 1980). As stocks of more desirable abalone species declined, a commercial fishery for black abalone developed in the late-1960s and peaked in the mid-1970s. In addition to fishing pressure, mainland coastal populations were also probably impacted by coastal development and pollution (Altstatt et al. 1996). In the mid-1980s, black abalone populations in the northern Channel Islands experienced mass mortalities that cannot be attributed to human activities; a wasting disease called withering syndrome (WS) appears to be the primary cause of the decline (Haaker et al. 1992; Altstatt et al. 1996). Withering syndrome continues to cause high mortality in black abalone populations and may now be spreading among mainland populations (Altstatt et al. 1996). At the present time, no significant California mainland populations are known to exist south of the Point Conception area.

Methods and Materials

Black abalone were collected from deep cracks and crevices in rocky habitat during minus tides between July 1997 and November 1998. Most animals were found in the high- to mid-intertidal zone (Morris et al. 1980). Collecting sites (Figure 1) included Vandenberg Ecological Reserve (located at Pt. Arguello, Santa Barbara County), Cambria and San Simeon (located in San Luis Obispo County), Big Creek Marine Ecological Reserve (located on the Big Sur coast), Carmel Point (located immediately south of the town of Carmel), Point Piños (Monterey County), and Scotts Creek (Santa Cruz County). C. S. Friedman (University of Washington) kindly provided additional samples from Carmel Point and Vandenberg Ecological Reserve sites to supplement our field collections.

Sampled individuals varied in size from approximately 3 cm–25 cm. Due to temporal variation in growth, size is not a reliable measure of age (Morris et al. 1980). Tissue samples were obtained (nondestructively) by removing the animal from the substrate, clipping a small piece of epipodium or a few epipodial tentacles and then replacing the animal on the substrate. Typical samples consisted of approximately 40 mg of tissue; samples were transported in liquid nitrogen and stored at -80°C. Protein electrophoresis methods were as described above for red abalone.

Results

Allelic frequencies for three polymorphic allozyme loci in the seven populations sampled are summarized in Table 3. Failure to resolve additional loci was due in part to the restricted nature of tissue available because of our nondestructive sampling of epipodial tissue. Significant heterogeneity in allelic frequencies among sites was detected at all three loci (Table 4). Although the most common allele did not differ among populations at any of the loci, the frequencies of other alleles varied widely. For example, the PGM^B allele, not observed among 60 individuals sampled at Scotts Creek, reached a frequency of 0.213 at Cambria (N = 54). Similarly, the $AAT\text{-}1^B$ allele, absent at Cambria, occurred at a frequency of 0.283 at Big Creek (N = 23). There was no pattern across populations regarding the absence of the less common alleles; of the eleven alleles observed in the study, at least nine were found in each population. Hence, even where field sampling located relatively few abalone (e.g., Big Creek), there was no clear evidence of genetic impoverishment.

Estimates of F_{ST} for each locus and the mean over all alleles are presented in Table 4 along with total sample sizes; values for AAT-1 and PGM were significantly different from zero and, like the heterogeneity of allelic frequencies, indicate significant population differentiation. Based on mean F_{ST}, Nm is estimated to be approximately 6.2, a value that suggests enough interpopulation exchange to prevent extensive population differentiation. However, there was no consistent relationship between geographic and genetic distances for the population comparisons. Interestingly, all the largest pairwise distances involved the Big Creek population, which is located in the middle of the sampled geographic range.

Discussion

Previous work on the genetic structure of natural populations of abalone has yielded variable results. The allozyme data collected here demonstrates significant genetic differentiation among *H. cracherodii* populations along the Central California coast. The observed mean F_{ST} value (0.039), is considerably higher than that observed in other abalone allozyme studies. Allozyme analyses of three *H. rufescens* populations (see Part One), yielded an estimate of F_{ST} of only 0.012, which was not significantly different from zero. Data on

other abalone species are typically consistent with this lower estimate of population subdivision (e.g., F_{ST} values of 0.022 and 0.014 for *H. rubra* and *H. laevigata*, respectively; Withler, 2000). Hence, population subdivision in *H. cracherodii* is more pronounced than that estimated with allozyme analyses in other abalone species studied to date.

Using F_{ST} values calculated from the allozyme data, levels of gene flow among *H. cracherodii* populations are estimated to be relatively high ($Nm > 4$ for even the most differentiated locus and $Nm > 6$ for the mean F_{ST}). However, as recently reviewed by Bossart and Prowell (1998), a large number of caveats surround the interpretation of F_{ST} and the corresponding estimates of Nm. In particular, such summary statistics fail to take into account various aspects of demographic and population genetic instability common to many study systems. Hence, analyses of the allelic frequency differences themselves may be as insightful as consideration of $F_{ST.}$ (Bossart and Prowell, 1998). In this regard, two aspects of the data merit special attention. First, the degree of differentiation in the frequencies of less common alleles is rather remarkable. Even with sample sizes of only 50 alleles, the absence of alleles present in frequencies of greater than 0.2 in other populations is highly significant and likely indicates considerably restricted gene flow among populations. Although the absence of alleles in a given population may reflect a founder event or historical population bottleneck (as suggested by the low mtDNA diversity), the lack of consistency across loci does not make this a strong hypothesis in the current case. Furthermore, even if the loss of alleles is due to a population bottleneck, absence of such alleles across the numerous cohorts represented in our sampling also points to a persistent restriction of inter-population gene flow.

Aside from the analyses presented here for black abalone, the only other studies of abalone population structure in North America concern the red abalone, *H. rufescens*. Although both Gaffney et al. (1996) and Burton and Tegner (2000) found minor allele frequency differences at the AAT-1 allozyme locus between samples of *H. rufescens* from Northern and Southern California, and no significant differences were observed at two other polymorphic allozyme loci. Furthermore, mtDNA sequence data from the COI gene (Burton and Tegner, 2000) and data on allelic frequencies at one microsatellite locus (Kirby et al. 1998) failed to find evidence for differentiation between populations of *H. rufescens* from Northern and Southern California. Hence, available evidence suggests that there are substantial differences in the population structure of the two California haliotids studied to date, with geographically more widespread sampling of red abalone showing less population genetic divergence than reported here for black abalone.

A partial explanation for the differences in population structure between abalone species may lie in the pattern of spawning. While *H. rufescens* spawn continuously throughout the year, other California haliotids, including *H. cracharodii*, are more seasonal (Boolootian et al. 1962). Black abalone spawn

primarily in the summer, when individuals lose about 20% of tissue wet weight. The longer spawning season means that *H. rufescens* larvae will experience a broader range of oceanographic conditions and might, therefore, have greater realized dispersal. Such dispersal, in turn, may account for the low genetic divergence among populations. In contrast, *H. cracherodii* larvae will experience a more limited range of oceanographic conditions during its reduced spawning season and consequently realize reduced dispersal (resulting in more population differentiation). Tegner (1993) invoked this explanation for observed differences in the recovery of abalone populations following the destruction of the kelp beds off the Palos Verdes peninsula; red abalone stocks showed significantly better recovery despite the fact that other species (pink and green abalone) were previously dominant at the site.

Previous investigations of the relationship between larval dispersal and the extent of population genetic differentiation in invertebrates have focused largely on the duration of the larval period (Burton, 1983; Hellberg, 1996) and the strength and patterns of ocean currents (Roberts, 1997). A role for larval behavior has also been widely recognized (Burton and Feldman, 1982). The comparison here between red and black abalone suggests that the duration of the spawning season may also impact a species' dispersal success. Although the genetic consequences of seasonal changes in larval dispersal patterns may be dramatic, they have previously received little attention.

The importance of spawning season on dispersal depends on the extent of temporal variation in ocean currents. Along the coast of California, seasonal changes in coastal oceanographic conditions are profound (Browne, 1994). Ongoing ocean current investigations in the Santa Barbara Channel–Santa Maria Basin Circulation Study (Center for Coastal Studies, Scripps Institution of Oceanography[2]) provide interesting data in this regard (Figure 2). The Santa Maria Basin lies near the center of our black abalone sampling range. Trajectories of drifters released in the basin in winter (December 1996) and summer (July 1997) can show substantial differences. In the case of the plotted trajectories, transport by currents in the summer (when black abalone spawn) would be quite limited compared to that observed in winter. Red abalone, which spawn throughout the year (with a peak in winter; Boolootian et al. 1962) would be expected to experience enhanced dispersal potential. These expectations are consistent with the three-fold higher levels of population differentiation (based on F_{ST} values) observed in black versus red abalone.

Despite their pelagic larval stages, ecological observations have previously indicated that pink *(H. corrugata*) and green (*H. fulgens*) abalone are poor dispersers/colonizers (Tegner, 1993). Based on our population genetic analysis, we suspect that black abalone, *H. cracherodii*, fit this pattern as well. In the two cases where we obtained samples within 12 km of one another, no genetic differentiation was observed, suggesting that effective dispersal and

[2]see website http://www-ccs.ucsd.edu/research/sbcsmb/drifters/

gene flow occurs on that spatial scale. Although the data on genetic differentiation are not consistent with extensive longer distance dispersal, the fact that most alleles and haplotypes are shared across all sampled populations suggests that these populations have a recent common ancestry or ongoing low levels of gene flow. This apparent discrepancy between allelic distributions and significant differences in their frequencies is likely due to differences in the time scales relevant to different processes—even low levels of dispersal may yield sufficient gene flow to spread alleles across the species range over long periods of time. Whether such spread is because of persistent low levels of larval exchange or rare episodic events cannot be distinguished with the present data. However, in either case, levels of dispersal are unlikely to provide sufficient recruitment for recovery of fishery stocks even over the course of decades. These inferences have two implications for the strategy of using marine protected areas to enhance abalone fishery resources. On the one hand, the data suggest that such protected areas may not be an effective source of recruits for the enhancement or re-establishment of distant populations; on the other hand, the data suggest that there may be sufficient local recruitment that populations isolated in protected areas can be self-sustaining. These inferences drawn from genetic data provide independent support for similar conclusions derived from combined consideration of abalone life history and empirical oceanographic studies (Tegner and Butler, 1985; Tegner, 1993).

Part Three

Genetic Studies of Purple Urchins (*Strongylocentrotus purpuratus*)

in Vandenberg and Big Creek Reserves

In order to assess the extent to which existing marine reserves harbor differentiated stocks of invertebrates, purple urchins of different size classes were collected from Vandenberg and Oystercatcher Point (neighboring Big Creek) and subjected to allozyme analysis (following the methods of Edmands et al. 1996). The data are summarized in Table 5. Although there is one statistically significant difference between the populations (pooling all size classes), most loci show more variation among size classes within populations than between populations. This result is similar to that observed by Moberg and Burton (2000) and is somewhat difficult to interpret beyond saying that the genetics of this system are more dynamic than might be predicted from extensive and continuing mixing of the larval pool. Work on this system is ongoing.

Figures

Figure 1. Map of coastal California sampling sites for Haliotis cracharodii.

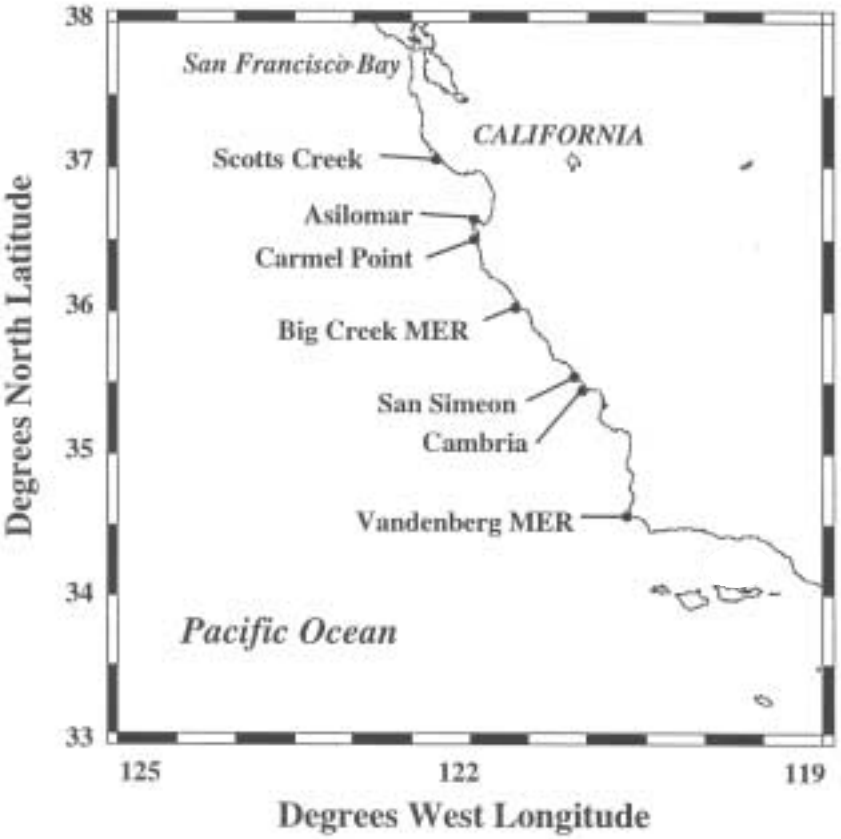
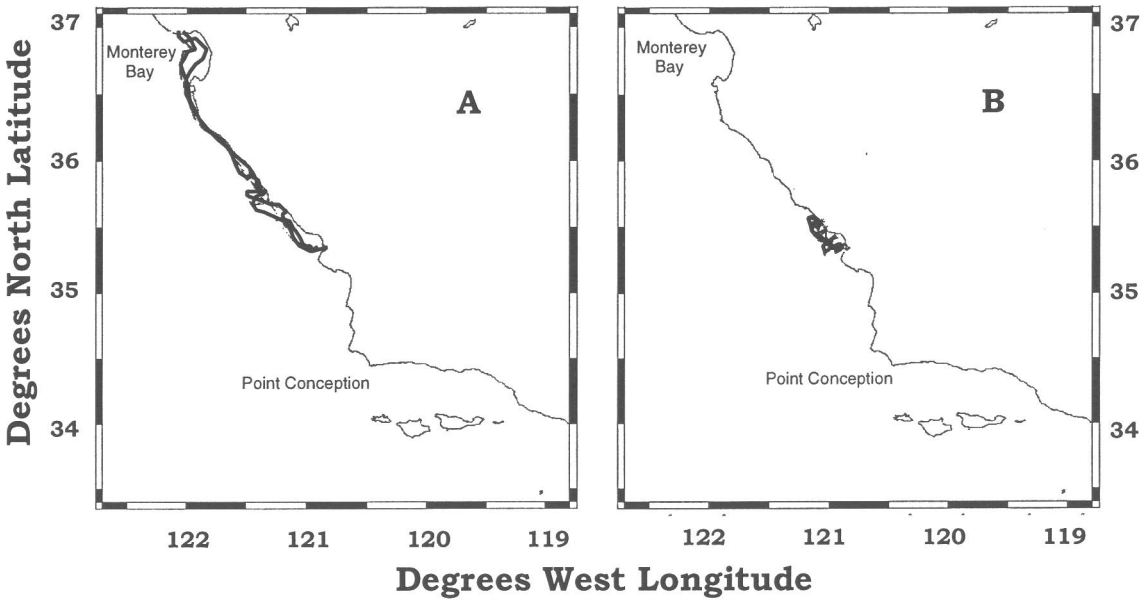


Figure 2. Trajectories of drifters released in the Santa Maria Basin: (A) December 1996, drifters 363-364 (each was tracked for 14 days); and (B) July 1997, drifters 419-420 (419 tracked for 38 days; 420 beached in 3 days).



Tables

Table1. *Haliotis rufescens*. Allozyme frequencies for three populations collected in 1999 compared to data reported in Gaffney et al. (1996). Allelic designations reflect increasing anodal migration. Because different electrophoretic systems were employed, allelic identities between this study and that of Gaffney et al. are inferred from frequency data. n = number of individuals scored.

		GPI					PGM							AAT1			
Allele	n	a	b	c	d	n	a	b	c	d	e	f	n	a	b	c	d
Data from this study																	
Population																	
SMI-99	46	0.913	0.076	0.011		46		0.033	0.076	0.674	0.207	0.011	43	0.174	0.826		
HSC-99	30	0.883	0.100	0.017		30		0.133	0.067	0.517	0.267	0.017	30	0.067	0.033	0.883	0.017
SH-99	59	0.847	0.127	0.026		55	0.009	0.100	0.073	0.618	0.200		48	0.083	0.906	0.010	
Data from Gaffney et al. (1996)																	
Population																	
SMI-92	60	0.500	0.500			60			0.008	0.775	0.217		60	0.025	0.975		
NC-92*	116	0.884	0.108	0.004	0.004	116	0.004	0.009	0.129	0.543	0.315		116	0.086	0.914		
SC-79*	208	0.858	0.132	0.007	0.002	192		0.010	0.130	0.594	0.266		84	0.030	0.970		

*Samples were pooled over multiple populations in NC (Northern California) or SC (Southern California)

Table 2. *Haliotis rufescens*. Cytochrome oxidase subunit 1 haplotypes and their frequencies in three California populations. Base positions sequenced (484 bases) correspond to positions 37—520 in the Genbank sequence (AF060837) determined b Metz et al. (1998).

	Position			
	11122	Population		
	9903433			
Haplotype	6958417	SMI	HSC	SH
1	AAAAAAA	8	5	5
2	AAAAAAG	4	6	6
3	AGAAGAA	3	5	6
4	AGAAGGA	1	1	4
5	AAGAAAA	0	1	0
6	GAGAAAA	3	2	1
7	AAAGAAG	1	1	0

Table 3. Allelic Frequencies for *Haliotis cracherodii* at seven sites in California. Population names are abbreviated as: Vandenberg Ecological Reserve(V), Cambria(Cam), San Simeon(SS), Big Creek Ecological Reserve(BC), Carmel Point(CP), Asilomar(Asl), Scotts Creek(SC).

		Population					
Locus Allele	V	Cam	SS	BC	CP	Asl	SC
PGI							
A	0.004						
B	0.021	0.043		0.021	0.049		0.022
C	0.949	0.931	0.952	0.938	0.923	0.968	0.946
D	0.025	0.026	0.048	0.042	0.028	0.032	0.033
2N	236	116	62	48	142	94	92
PGM							
A	0.610	0.593	0.638	0.625	0.635	0.737	0.842
B	0.009	0.213	0.121	0.146	0.151	0.059	
C	0.326	0.157	0.155	0.188	0.167	0.144	0.125
D	0.055	0.037	0.086	0.042	0.048	0.059	0.033
2N	236	108	58	48	126	118	120
AAT-1							
A	0.744	0.707	0.714	0.413	0.757	0.807	0.620
B	0.074		0.071	0.283	0.110	0.016	0.121
C	0.182	0.293	0.214	0.304	0.132	0.177	0.259
2N	242	116	56	46	136	124	116

Table 4. Results of contingency table tests of heterogeneity of allelic counts (G) and estimates of Fst and Nm for all loci.

Locus :	PGI	PGM	AAT-1	Mean
G :	34.284	105.735	65.915	
df :	18	18	12	
p :	0.012	0.000	0.000	
Fst:	0.009	0.032	0.054	0.039
Nm :	27.562	7.498	4.400	6.178
2N :	790	814	836	813
P :	0.50	<0.001	<0.001	<0.001

Table 5. *Strongylocentrotus purpuratus*. Allele frequencies and sample sizes (N = mean number of individuals scored per locus) for pooled samples (p), adults (a), juveniles (j), and recruits (r) from Big Creek Ecological Reserve (BC) and Vandenberg Ecological Reserve (V).

Locus, allele	Location (size class)						
	BC	V	BC		V		
	(p)	(p)	(j)	(r)	(a)	(j)	(r)
Got-1							
107	0.000	0.003	0.000	0.000	0.000	0.010	0.000
105	0.074	0.059	0.083	0.057	0.083	0.077	0.027
103	0.421	0.199	0.450	0.371	0.319	0.221	0.100
100	0.353	0.364	0.342	0.371	0.347	0.327	0.409
97	0.147	0.294	0.117	0.200	0.181	0.279	0.382
90	0.005	0.080	0.008	0.000	0.069	0.097	0.082
Got-2							
107	0.046	0.019	0.009	0.095	0.014	0.020	0.023
103	0.325	0.447	0.327	0.321	0.257	0.275	0.407
100	0.582	0.634	0.627	0.524	0.622	0.696	0.570
97	0.046	0.034	0.036	0.060	0.108	0.010	0.000
Idh							
102	0.000	0.008	0.000	0.000	0.025	0.000	0.000
100	0.505	0.504	0.578	0.420	0.450	0.654	0.434
98	0.241	0.337	0.336	0.130	0.313	0.256	0.415
94	0.236	0.125	0.078	0.420	0.188	0.077	0.113
92	0.019	0.027	0.009	0.030	0.025	0.013	0.038
Mpi							
110	0.022	0.016	0.016	0.029	0.032	0.000	0.017
105	0.147	0.103	0.131	0.167	0.149	0.082	0.083
100	0.491	0.381	0.492	0.490	0.330	0.439	0.375
95	0.188	0.324	0.197	0.176	0.351	0.255	0.358
92	0.076	0.093	0.082	0.069	0.096	0.102	0.083
90	0.071	0.074	0.074	0.069	0.043	0.102	0.075
88	0.004	0.010	0.008	0.000	0.000	0.020	0.008
Pgi							
109	0.000	0.018	0.000	0.000	0.010	0.036	0.008
105	0.301	0.314	0.310	0.290	0.306	0.304	0.328
100	0.677	0.660	0.675	0.680	0.663	0.652	0.664
98	0.022	0.009	0.016	0.030	0.020	0.009	0.000
Pgm							
105	0.017	0.007	0.016	0.020	0.021	0.000	0.000
103	0.057	0.031	0.055	0.059	0.021	0.060	0.011
100	0.535	0.441	0.531	0.539	0.490	0.490	0.337
97	0.300	0.344	0.297	0.304	0.396	0.310	0.326
95	0.065	0.146	0.078	0.049	0.063	0.100	0.283
93	0.026	0.028	0.023	0.029	0.010	0.040	0.033
91	0.000	0.003	0.000	0.000	0.000	0.000	0.011
N	106.7	145.8	60.2	46.5	53.5	49.5	42.8

Acknowledgments

We thank P. B. Edwards, C. S. Friedman, P. L. Haaker, I. Tanaguchi and L. I. Vilchis for obtaining the samples, and the Pew Fellows Program in Marine Conservation for its financial support (to M. J. Tegner). T. Ballard provided laboratory assistance. This work was completed under the sponsorship of the California Department of Fish and Game, Marine Resources Protection Act—Marine Ecological Reserves Research Program administered by California Sea Grant College Program under grant number FG-5335MR, project number R/M-1. D. E. Hamm also received partial support from a Sea Grant Traineeship under NOAA grant number NA66RG0477, project number R/F-170. Work was completed as of August 31, 1999.

References

Altstatt JM, Ambrose RF, Engle JM, Haaker PL, Lafferty KD, Raimondi PT (1996) Recent declines of black abalone Haliotis cracherodii on the mainland coast of Central California. Mar Ecol Prog Ser 142:185–192.

Boolootian R, Farmanfarmaian AA, Giese AC (1962) On the reproductive cycle and breeding habits of two western species of *Haliotis*. Biol Bull 122:183–193.

Bossart JL, Prowell DP (1998) Genetic estimates of population structure and gene flow: Limitations, lessons and new directions. Trends in Ecol Evol 13:202–206.

Browne DR (1994) Oceanic circulation in and around the Santa Barbara Channel. In Halvorson WL, Maender GJ (eds.). The fourth California Islands symposium: Update on the status of resources. Santa Barbara Museum of Natural History, Santa Barbara, California, pp. 27–34.

Burton RS (1983) Protein polymorphisms and genetic differentiation of marine invertebrate populations. Mar Biol Let 4:193–206.

Burton RS, Feldman MW (1982) Population genetics of coastal and estuarine invertebrates: Does larval behavior influence population structure? In Kennedy VS (ed.), Estuarine Comparisons, Academic Press, N.Y., pp. 537–551.

Burton RS, Tegner MJ (2000) Enhancement of red abalone (*Haliotis rufescens*) stocks at San Miguel Island: Reassessing a success story. Mar Ecol Prog Ser 202:303–308.

Cole, MA, Morgan RP (1978) Genetic variation in two populations of blue crab, *Callinectes sapidus*. Estuaries 1:202–205.

Edmands S, Moberg P, Burton RS (1996) Allozyme and mitochondrial DNA evidence of population subdivision in the purple sea urchin, *Stronglyocentrotus purpuratus*. Mar Biol 126:443–450.

Gaffney PM, Rubin VP, Hedgecock D, Powers DA, Morris G, Hereford L (1996) Genetic effects of artificial propagation —signals from wild and hatchery populations of red abalone in California. Aquaculture 143:257–266.

Haaker PL (1994) Assessment of abalone resources at the Channel Islands. In Halvorson WL, Maender GJ (eds.), The fourth California Islands symposium: Update on the status of resources. Santa Barbara Museum of Natural History, Santa Barbara, CA, pp. 83–95.

Haaker PL, Parker DO, Togstad H, Richards DV, Davis GE, Friedman CS (1992) Mass mortality and withering syndrome in black abalone, *Haliotis cracherodii*, in California. In Shepherd SA, Tegner MJ, Guzman del Proo SA (eds.), Abalone of the world: biology, fisheries and culture, Fishing News Books, Oxford. pp. 214–224.

Hamm, D.E., and R.S. Burton. 2000. Population genetics of black abalone, *Haliotis cracherodii*, along the central California coast. J Exp Mar Biol Ecol. 254:235–247.

Harris H, Hopkinson DA (1976) Handbook of Enzyme Electrophoresis in Human Genetics. North-Holland, Amsterdam.

Hellberg ME (1996) Dependence of gene flow on geographic distance in two solitary corals with different larval dispersal capabilities. Evolution 50:1167–1175.

Hudson, RR, Slatkin M, Maddison WP (1992) Estimation of levels of gene flow from DNA sequence data. Genetics 132:583–589.

Jiang L, Wu WL, Huang PC (1995) The mitochondrial DNA of Taiwan abalone *Haliotis diversicolor* Reeve, 1846 (Gastropoda: Archaeogastropoda: Haliotidae). Molec Mar Biol Biotech 4:353–364.

Kirby VL, Villa R, Powers DA (1998) Identification of microsatellites in the California red abalone, *Haliotis rufescens*. J Shellfish Res 17:801–804.

Kordos LM, Burton RS (1993) Genetic differentiation of Texas Gulf coast populations of the blue crab, *Callinectes sapidus*. Mar Biol 117:227–233.

Lynch M, Crease TJ (1990) The analysis of population survey data on DNA sequence variation. Mol Biol Evol 7: 377–394.

McMillen-Jackson, AL, Bert TM, Steele P (1994) Popultion genetics of the blue crab *Callinectes sapidus*: Modest population structuring in a background of high gene flow. Mar Biol 118:53–65.

Metz EC, Robles-Sikisaka R, Vacquier, VD (1998) Nonsynonymous substitution in abalone sperm fertilization genes exceeds substitution in introns and mitochondrial DNA. Proc Natl Acad Sci USA 95:10676–10681.

Moberg PE, Burton RS (2000) Genetic heterogeneity among adult and recruit red sea urchins, *Strongylocentrotus franciscanus*. Mar Biol 136:773–784.

Morris RH, Abbott DP, Haderlie EC (1980) Intertidal Invertebrates of California. Stanford University Press, Stanford, CA.

Munroe JL, Bell JD (1997) Enhancement of marine fisheries resources. Rev Fish Sci 5:185–222.

Nei M (1982) Evolution of human races at the gene level. In Bonne-Tamir B, Cohen T, Goodman RM (eds.), Human genetics, part A: The unfolding genome. Alan R. Liss, New York, pp. 167–181.

Roberts CM (1997) Connectiviy and management of Caribbean coral reefs. Science 278:1454–1457.

Rozas J, Rozas R (1999) DnaSP version 3: An integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics 15:174–175.

Schiel DR (1993) Experimental evaluation of commercial-scale enhancement of abalone *Haliotis iris* populations in New Zealand. Mar Ecol Prog Ser 97:167–181.

Shepherd SA, Brown LD (1993) What is an abalone stock: Implications for the role of refugia in conservation. Can J Fish Aquat Sci 50:2001–2009.

Swofford DL (1999) PAUP (Phylogenetic Analysis using Parsomony) version 4. Sinauer Associates, Sunderland, MA.

Tegner MJ (1993) Southern California abalones: Can stocks be rebuilt using marine harvest refugia? Can J Aquat Sci 50:2010–2018.

Tegner MJ (2000) Abalone (*Haliotis* spp.) enhancement in California: What we've learned and where we go from here. Can Spec Pub Fish Aquat Sci 130:61–71.

Tegner MJ, Butler RA (1985) Drift tube study of the dispersal potential of green abalone (*Haliotis fulgens*) larvae in the Southern Californian bight: Implications for recovery of depleted populations. Mar Ecol Prog Ser 26:73–84.

Tegner MJ, Butler RA (1989) Abalone seeding. InHahn K (ed.), Handbook of culture of abalone and other marine gastropods. CRC Press, Boca Raton, FL. Pp. 157–182.

Waples RS (1987) A multispecies approach to the analysis of gene flow in marine shore fishes. Evolution 41:385–400.

Withler RE (2000) Genetic tools for identification and conservation of exploited abalone species. Can Spec Pub Fish Aquat Sci 130:101–110.

Yeh FC, Boyle TJB (1997) Population genetic analysis of co-dominant and dominant markers and quantitative traits. Belg J Botany 129:157.